DRG

DRG® Leptospira IgM (EIA-4715)

CE IVD

Revised 15 June 2007 **RUO in the USA**

INTENDED USE

The DRG Leptospira IgM ELISA test is an enzyme immunoassay for the detection of antibodies to Leptospira biflexa (serovar patoc 1) for the serological confirmation of infections in serum, and plasma.

This test is intended to be performed by trained laboratory personnel only. In the United States, this kit is intended for **Research Use Only.**

SUMMARY AND EXPLANATION

The clinical manifestations of leptospirosis range from a mild catarrh-like illness to icteric disease with severe liver and kidney involvement. Natural reservoirs for leptospirosis include rodents as well as a large variety of domesticated mammals. The organisms occupy the lumen of nephritic tubules in their natural host and are shed into the urine. Human infection derives from direct exposure to infected animals (veterinarians, abattoir workers, or dairy workers for example) or by exposure to environments contaminated by animal carriers (e.g. agricultural workers). Bathing or swimming in water sources about which livestock have been pastured has been demonstrated to be a potential infection hazard. The organisms enter the host through skin abrasions, mucosal surfaces or the eye.

The incubation period can range from 3 to 30 days but is usually found to be 10 to 12 days. Antibodies can become detectable by the 6th to 10th day of disease and generally reach peak levels within 3 to 4 weeks. Antibody levels then gradually recede but may remain detectable for years.

Epidemiologic factors, clinical findings, exposure in endemic regions, and other laboratory results should be considered in diagnosing acute disease. Acute disease diagnosis will also include a positive laboratory confirmation in many cases.

This test is designed to measure acute infections with leptospira. Confirmation of a positive sample by additional methods should be followed.

ASSAY PRINCIPLE

The microwells are coated with purified leptospira Patoc 1 antigen.

During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethlybenzidine, or TMB) is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

REAGENTS - MATERIALS PROVIDED

1. **Test strips**:
   Microwells containing leptospira antigen -96 test wells in a test strip holder.
2. **Enzyme Conjugate**:
   One (1) bottle containing 11 ml of anti-human IgM antibody conjugated to peroxidase.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

3. **Positive Control Serum**:
   One (1) vial containing 1 ml of diluted positive IgM human serum.
4. **Negative Control Serum**:
   One (1) vial containing 1 ml of diluted negative human serum.
5. **Chromogen**:
   One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **RF Absorbent**:
   One (1) bottle containing 5 ml of goat anti-human IgG.
7. **Wash Solution Concentrate** (20X):
   One (1) bottle containing 25 ml of concentrated buffer and surfactant.
8. **Dilution Buffer**:
   Two (2) bottles containing 30 ml of buffered protein solution.
9. **Stop Solution**:
   One (1) bottle containing 11 ml of 1 M phosphoric acid.

STATEMENT OF WARNINGS

Do not use solutions if they precipitate or become cloudy.

Wash concentrate may show crystallization upon storage at 2-8º C. Crystallization will disappear after dilution to working strength.

Dilution buffer is a colloidal solution. It will appear opaque and have a precipitate form.

Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.

Treat all sera as if capable of being infectious. Negative control and positive control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods.

This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

Do not add azide to any of the kit reagents.

STORAGE

Reagents, strips and bottled components: Store between 2 -8º C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Warning -Potential Bio-Hazardous Material

Sera used for positive and negative controls (available separately) are tested by an FDA approved method for the presence of the antibody to human immunodeficiency virus (HIV) as well as for hepatitis B surface antigen and found to be negative (not repeatedly reactive). Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, specimens as well as controls should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.

SPECIMEN COLLECTION AND HANDLING

The DRG Leptospira IgM ELISA test should be performed on serum.

Serum may be stored at 2-8 °C for up to five days. Serum may be frozen below -20 °C for extended periods. Freezing whole blood samples is not advised.

Single specimens are used to assess exposure; two specimens collected at different times from the same individual are used to show sero-conversion. Paired specimens should be tested at the same time. It is recommended that a convalescent specimen be collected from patients showing either an initially non-reactive result or a weakly reactive result.

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes

Squeeze bottle for washing strips (narrow tip is recommended)

Reagent grade water and graduated cylinder

Tubes for sample dilution

Absorbent paper

Suggested Materials

ELISA plate reader with a 450 nm and a 620 -650 nm filter (optional if results are read visually)

PROCEDURE

Wash Buffer

Remove cap and add contents of Wash Solution concentrate bottle to 475 ml of reagent grade water.

Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling. Avoid generating bubbles in the wells during the washing steps.

Samples

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used within five days.

Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples:

Make a **1:40 dilution** of patients' sera using the Dilution Buffer (e.g. 10 µl sera and 390 µl Dilution Buffer).

PERFORMANCE OF TESTS

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.

2. **NOTE: Negative and positive controls are supplied pre-diluted. Do not dilute further.**
   Do ot add RF Absorbent to negative and positive controls. Transfer 100 µl negative and positive controls to wells (#1, #2 respectively) after initial five minute incubation.
   Dilute patient sera 1:40 in Dilution Buffer. **To 100 µl of diluted serum add 40 µl of RF Absorbent**. **Mix well. Incubate in a tube for 5 minutes.**
   Transfer all 140 µl of test samples to the remaining wells (#3-#96).
3. Incubate at room temperature (15 to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Slap wells against paper towels to remove all liquid.
9. Add 2 drops of the Chromogen to every well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of the Stop Solution and mix by tapping strip holder.
12. Read within one hour of adding Stop Solution.

READING RESULTS

Visually:

Look at each well against a white background (e.g. paper towel) and record as clear or +,++ or +++ reaction.

ELISA Reader:

Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

QUALITY CONTROL

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range.

Expected values for the controls are: **Negative** 0.0 to 0.3 OD units
Positive 0.5 OD units and above

TROUBLESHOOTING

Negative control has excessive color after development.

Reason: inadequate washings

Correction: wash more vigorously. Remove excessive liquid from the wells by tapping against an Absorbent towel. Do not allow test wells to dry out.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

INTERPRETATION OF THE TEST

Initially Non-reactive:

Samples interpreted as non-reactive (0.0-0.3 OD units, or zero color) indicate antibody is not present in the sample. Since antibody may not be present during early disease, (5-8 days incubation), confirmation 2-3 weeks later is indicated for laboratory diagnosis. At this later time, patients showing weak reactions (0.5 - ≤1.0 or +, ++) should be further tested by alternate methods or re-tested 10-14 days later. A convalescent serum with a significant reaction (>1.0 OD) indicates the formation of specific antibody against leptospira. An initially negative result followed by a positive result implies seroconversion.

Initially Weakly Reactive:

Weakly reactive specimens should be cautiously interpreted. In normal populations, weakly reactive samples are infrequent but possible. Confirmation using a sample collected 2-3 weeks later (paired acute and convalescent sera) is recommended. >1.0 OD in the second sample confirms the presence of recent, specific antibody. [Caution: If this is a cross-reactive antibody, the convalescent serum sample may not show a higher antibody level than the acute sample.]

If sample reading remains at ≥ 0.5 - ≤1.0 OD, or +, ++, a second methodology should be considered, or the sample may be interpreted as taken beyond rising titer (titer declining).

Initially Reactive:

Samples interpreted as strongly reactive (>1.0 OD or +++ or >) may indicate the presence of specific antibody. Antibody presence alone cannot be used for diagnosis of acute infection, since antibodies from prior exposure may circulate for a prolonged period of time.

LIMITATIONS OF THE PROCEDURE

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

The ELISA has been tested against many serovars, but cannot guarantee that all strains will react equally.

Do not use in veterinary samples.

Treatment is often indicated prior to completion of serologic diagnosis, which requires at least two weeks. Acute leptospirosis must be treated immediately and should not wait for serological confirmation. Diagnosis of leptospira infection should not be made based on results of the ELISA test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.

Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

Many strains and serovars of leptospira are known. Many of the strains are geographically dominant in some areas and not in others. Biflexa Patoc 1 is known to cross react with most serovars **but usually does not cross-react with animal strains**. The relative strength of the reactions may vary by antigen. This must be considered during interpretation. Use of culture or the MAT test is recommended for confirmation as these tests are serovar specific.

Since serological assay methods may yield different results for weakly reactive samples, a second serological method (i.e. an alternative method that separately identifies IgM and IgG antibody) is recommended.

EXPECTED VALUES

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

Antibody levels are generally low or absent during very early infection. Symptomatic patients may have no antibody during the first 1-2 weeks after exposure and the antibody titer will rise with time.

PERFORMANCE CHARACTERISTICS

Specific performance data is available upon request.

REFERENCES

1. Kelly PW. Leptospirosis. In, Infectious disease in medicine and surgery. Gorbach S, Bartlett J, Balcklow N, (eds): Philadelphia, Saunders, 1991, pp.1295-1302.
2. Ribeiro MA, Assis CSN, Romero EC. Serodiagnosis of human leptospirosis employing immunodominant antigen. Serodiagn. Immunother. Infect. Disease 1994; 6:140-144.
3. Turner LH. Leptospirosis II. Trans. Royal Soc. Trop. Med. & Hygiene 1968; 62:880-889.
4. Pappas MG, Ballou WR, Gray MR, et. al. Rapid serodiagnosis of leptospirosis using the IgM-specific dot-ELISA: Comparison with the microscopic agglutination test. Am J Trop Med Hyg 1985; 34(2):346-354.
5. Alder B, Murphy AM, Locarnini SA, et.al. Detection of specific anti-leptospiral immunoglobulin M and immunoglobulin G in human serum by solid phase enzyme-linked immunosorbent assay. J. Clin. Micro 1980; 11:452-457.
6. Cinco M, Balanzini D, Banfi E. Evaluation of an immunoenzymatic test (ELISA) for the diagnosis of leptospirosis in Italy. Eur. J. Epidemiology 1992; 8:677-682.
7. Ribeiro MA, Sousa CC, Almeida SHP. Dot-ELISA for human leptospirosis employing immunodominant antigen. J. Trop.Med. Hyg. 1995; 98:452-456.
8. Gussenhoven GC, van der Hoorn M, Goris M, et. al. LEPTO dipstick, a dipstick assay for detection of leptospiraspecific immunoglobulin M antibodies in human sera. J. Clin. Micro. 1997; 35(1):92-97.
9. Watt G, Alquiza LM, Padre LP, et. al. The rapid diagnosis of leptospirosis: A prospective comparison of the dot enzyme-linked immunosorbent assay and the genus-specific microscopic agglutination test at different stages of illness. J. Infect. Dis. 1988; 157(4)840-842.
10. Levett PN, Branch SL, Paxton H. Prospective Evaluation of Dot-ELISA Method for Detection of Acute Leptospirosis. Abstract of the 37th ICAAC (ASM) 1997; D-4: 83.

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com

SYMBOLS USED WITH DRG ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής

CE IVD

Revised 15 June 2007

RUO in the USA

Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

Version 070615

Fax: (908) 233-0758 • E-mail: corp@drg-international.com •Web: www.drg-international.com